AN IN VITRO PROTOCOL FOR HIGH FREQUENCY PLANT REGENERATION FROM COTYLEDONARY NODES OF Gerbera jamesonii L.

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Abstract-The present investigation deals with in vitro propagation of gerbera, a popular cut flower of Asteraceae family. Cotyledonary node explants were isolated from in vitro grown seedlings of five gerbera cultivars and were cultured on MS medium containing different combinations of cytokinins. Effect of genotypes and growth regulators on various shoot parameters was investigated. Experiment revealed that both genotype and growth hormone had significant effect on multiple shoot formation. Yellow gerbera cultivar and BAP-1 mg L⁻¹ performed better in multiple shooting. Micro shoots were subjected to auxin (NAA) treatment to induce roots and different concentration of NAA had been applied; NAA–5.0 mg L⁻¹ produced maximum number of roots. Well developed plantlets were transferred ex vitro and for hardening a numbers of media was used, among them mixture of coarse sand garden soil (1:1) was most promising for all the genotypes studied. This standardize protocol could be extrapolated for gerbera breeding and for large scale multiplication of plants.

Key words: Gerbera, cotyledon, in vitro, regeneration, hardening.

I. INTRODUCTION
Floriculture industry has been flourishing in the developing countries due to low cost of maintenance including low labor cost [1]. Gerbera (Gerbera jamesonii L.), one of the most popular cut flower of the world [2], was introduced in Bangladesh around two decades ago and the prevailing climatic condition has been proved suitable for its cultivation in Bangladesh. Initially gerbera was cultivated in a limited area, but recently its cultivation has been spread over vast areas. Now gerbera is considered as a high value flower crop. In light of global demand, gerbera could be a major source of foreign exchange as one of the non-traditional export items in Bangladesh [3]. Opportunity of gerbera cultivation is limited due to inadequate supply of new varieties and quality planting materials.

Gerbera is conventionally propagated by division of clumps. Sexual propagation by seeds has also been practiced in many countries. Seed propagation system offers an opportunity to create variation among the progeny that can lead to selection of new genotypes. For commercial gerbera cultivation in Bangladesh, priority is to be given equally in two dimensions: (i) development of mass propagation system for true to type plant propagules and (ii) development of breeding system for creation of new genotypes with lucrative colors. Mass propagation system through vegetative propagation or through in vitro clonal propagation has been described by many workers [4, 5, 6]. Traditional seed propagation has also been described by few researchers [7] and according to them seed
The propagation system has some limitations, because seeds of gerbera are very sensitive to germination in natural conditions and germination frequencies are not appreciable. Contrary to this in vitro seed germination followed by micropropagation may offer an alternative way for successful breeding and propagation of gerbera. In this system multiple shoot formation from a single seed is a very common phenomenon, whereas in traditional system initially one seed lead to produce a single plantlet. Tissue culture system has already been proven as healthy and mass propagation system in many plant species including gerbera. But in gerbera this technique is almost confined to vegetative clonal propagation by using shoot tips, capitulum, leaf midrib etc. [2].

So, an attempt was taken to develop a protocol for in vitro propagation of gerbera by using cotyledonary nodes of in vitro grown seedlings with the following specific objectives:

i. To optimize concentrations and combinations of growth regulators for shoot multiplication from cotyledonary nodes in five gerbera genotypes.
ii. To select proper level of NAA for root induction in regenerated plantlets.
iii. To choose the appropriate substrate for ex vitro survivability of in vitro grown plantlets.

II. MATERIALS AND METHODS

Plant material
Mature biological seeds of five genotypes viz. white, red, pink, magenta and yellow flower colored gerbera were collected from the ‘Gerbera Research Center’ of Agrotechnology Discipline, Khulna University.

Explantation and multiple shooting
The seeds were surface sterilized following conventional method using 70% ethanol and 0.2% mercuric chloride and rinsing them thrice in autoclaved double distilled water. Sterilized seeds were cultured onto hormone free MS [8] medium and the cultures were incubated in growth chamber, maintaining a 16 hours of photoperiod with a temperature of 25±1 °C. After 3 weeks of culture cotyledonary nodes (shoot tip with cotyledons) from germinated seedlings were isolated and inoculated onto multiple shoot inducing media fortified with various combinations of cytokinins viz. (i) No cytokinins, (ii) 6-Benzylaminopurine (BAP)-1.0 mg L\(^{-1}\), (iii) Kinetin (Kin) 1.0 mg L\(^{-1}\) and (iv) BAP -0.05 mg L\(^{-1}\) + Kin -1.0 mg L\(^{-1}\). Sub-cultures on same media were done twice at four weeks interval.

Rooting of multiple shoots
The multiple micro shoots produced were separated aseptically and transferred onto rooting media, (i) ½ MS + NAA- 0.0 mg L\(^{-1}\), (ii) ½ MS + NAA-2.5 mg L\(^{-1}\), (iii) ½ MS + NAA-5.0 mg L\(^{-1}\) and (iv) ½ MS + NAA-7.5 mg L\(^{-1}\).

Hardening of regenerated plants
The well rooted regenerated plantlets were hardened by transferring those to a small pot containing various substances, (i) Rice husk + vermi tea, (ii) Rice husk charcoal chip + vermi tea, (iii) Brick chip + vermi tea, (iv) Coarse sand + vermi tea, (v) Fine sand + vermi tea, (v) Coarse sand + vermi compost (1:1) and (vi) Coarse sand + garden soil (1:1). All the potting materials were sterilized by autoclaving before planting.

Data collection and analysis
Data on the following parameters were collected: (i) Number of shoots, (ii) Length of shoots, (iii) Number of leaves, (iv) Number of roots and (vi) Length of roots. The collected data were analyzed by using Analysis of Variance technique with the help of computer package (MSTAT-C) and the mean differences were adjudged with Duncan’s New Multiple Range Test [9].

III. RESULTS AND DISCUSSION
The study was conducted with a view to develop a protocol for high frequency plant regeneration from cotyledonary node explants. Results are presented and discussed bellow.
Shoot multiplication in vitro
Cotyledonary nodes of five gerbera genotypes were isolated from in vitro grown seedlings and were inoculated in culture bottle containing MS medium supplemented with different combinations of cytokinins. Performance of genotypes, growth regulators and their combined effect on shoot multiplication had been observed.

Effect of genotypes
A considerable range of variation with respect to genotype was noticed in all parameters studied for shoot multiplication. Yellow genotype produced maximum number of shoots (5.333) which was statistically similar with pink (5.167). White genotypes had the lowest shoot numbers (3.417). Shoot length varied from 8.721 to 25.140 mm. The highest shoot length (25.14 mm) was recorded in magenta genotypes and the lowest (8.721 mm) in pink genotype. Maximum number of leaves (8.583) was obtained from yellow gerbera and the lowest was found in red (5.917) genotypes which was statistically similar with white (5.833) genotypes.

Table 1. Effect of gerbera genotypes on shoot multiplication in vitro

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Shoot numbers</th>
<th>Shoot length</th>
<th>Leaf numbers</th>
</tr>
</thead>
<tbody>
<tr>
<td>White</td>
<td>3.417c</td>
<td>22.569b</td>
<td>5.833b</td>
</tr>
<tr>
<td>Red</td>
<td>3.833bc</td>
<td>13.095d</td>
<td>5.917b</td>
</tr>
<tr>
<td>Pink</td>
<td>5.167a</td>
<td>8.721e</td>
<td>6.917ab</td>
</tr>
<tr>
<td>Magenta</td>
<td>4.667ab</td>
<td>25.140a</td>
<td>7.250ab</td>
</tr>
<tr>
<td>Yellow</td>
<td>5.333a</td>
<td>14.811c</td>
<td>8.583a</td>
</tr>
</tbody>
</table>

LS

Data recorded at 30 days of inoculation of explants; *= Significant at 5% level; LS= Level of Significance

Effect of plant growth regulators (PGR) on shoot multiplication
Effect of PGR on shoot growth parameters was found variable. Medium supplemented with BAP @ 1 mg L\(^{-1}\) was identified best for the number of shoots (5.2) per culture. Leaf number did not differ significantly with the various PGR supplantations and ranged from 6.467 to 8.2.

Plant height was varied from 22.686 mm to 11.581 mm. The highest plant height (22.686 mm) was obtained when explants were cultured in MS media fortified with BAP @ 0.5 mg L\(^{-1}\) and Kin @ 0.5 mg L\(^{-1}\) and the lowest (11.581 mm) in PGR free medium.

Table 2. Effect of plant growth regulators on shoot multiplication in vitro

<table>
<thead>
<tr>
<th>PGR (mg L(^{-1}))</th>
<th>Shoot numbers</th>
<th>Shoot length (mm)</th>
<th>Leaf numbers</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAP 0.0</td>
<td>4.73ab</td>
<td>11.58d</td>
<td>6.53</td>
</tr>
<tr>
<td>1.0</td>
<td>5.20a</td>
<td>14.99c</td>
<td>8.20</td>
</tr>
<tr>
<td>0.0</td>
<td>4.00 b</td>
<td>18.21b</td>
<td>6.40</td>
</tr>
<tr>
<td>0.5</td>
<td>4.00 b</td>
<td>22.68a</td>
<td>6.47</td>
</tr>
</tbody>
</table>

LS

Data recorded at 30 days of inoculation of explants
PGR=plant growth regulators; *= Significant at 5% level; NS= Non-significant; LS= Level of Significance

BAP @ 1 mg L\(^{-1}\) gave best results for shoot induction [10]. Work on three gerbera genotypes (red, yellow and white) revealed that shoot multiplication occurred in all the three genotype of gerbera when MS was supplemented with BAP and NAA but shoots become stunted [3].

The response and extent of multiple shooting from cotyledonary nodes was dependent on the genotype and the growth regulators used in the medium. Such types of response had been reported in many crops [11] including gerbera.

Rooting of micro shoots
Well developed micro shoots were transferred to rooting media containing ½ MS with various level of NAA. Effect of NAA concentrations on rooting was found significant. The root numbers varied from 2.0 to 7.667. It was noticed that root numbers as well as root length were increased with increasing NAA concentration (Table 3).
Table 3. Effect of NAA concentrations on rooting of micro shoots

<table>
<thead>
<tr>
<th>Rooting hormone</th>
<th>Root number (Root length (mm))</th>
</tr>
</thead>
<tbody>
<tr>
<td>NAA-0.0 mgL⁻¹</td>
<td>2.000c 19.06b</td>
</tr>
<tr>
<td>NAA-2.5 mgL⁻¹</td>
<td>4.167b 21.69a</td>
</tr>
<tr>
<td>NAA-5.0 mgL⁻¹</td>
<td>7.667a 21.52a</td>
</tr>
<tr>
<td>LS</td>
<td>*</td>
</tr>
</tbody>
</table>

*= Significant at 5% level; LS= Level of Significance

Naz et al. (2012) also observed that higher concentration of NAA produced maximum roots on micro shoots. Although many authors reported that IBA was the best medium for root induction in Gerbera [3, 12].

Proper hardening of the germinated plants is essential for success in the propagation of ornamental plants. For hardening of the regenerated plants different media viz. (i) rice husk charcoal chip + vermi tea, (ii) rice husk charcoal chip + vermi tea, (iii) rice husk charcoal chip + vermi tea, (iv) brick chip + vermi tea, (v) coarse sand + vermi tea, (vi) coarse sand + garden soil (1:1) and (vii) brick chip + vermi compost (1:1). All the gerbera genotypes showed better performance in coarse sand and garden soil mixture.

IV. ACKNOWLEDGEMENT

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V. REFERENCES


Figure 1. Sequential steps of in vitro propagation of gerbera. A. Flower, B. Seeds, C. In vitro germination, D. Shoot multiplication, E. Data recording, F. Rooting, G. Ex vitro plant.

Hardening of regenerated plant

For hardening of regenerated plants different media were used viz. (i) rice husk + vermi tea, (ii) rice husk charcoal chip + vermi tea, (iii) rice husk charcoal chip + vermi tea, (iv) brick chip + vermi tea, (v) coarse sand + vermi tea, (vi) coarse sand + garden soil (1:1) and (vii) brick chip + vermi compost (1:1). All the gerbera genotypes showed better performance in coarse sand and garden soil mixture.
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